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Targeted Stimulation of Meiotic Recombination

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Summary

Meiotic recombination in *Saccharomyces cerevisiae* is initiated by programmed DNA double-strand breaks (DSBs), a process that requires the Spo11 protein. DSBs usually occur in intergenic regions that display open chromatin accessibility, but other determinants that control their frequencies and non-random chromosomal distribution remain obscure. We report that a Spo11 construct bearing the Gal4 DNA binding domain not only rescues *spo11Δ* spore inviability and catalyzes DSB formation at natural sites but also strongly stimulates DSB formation near Gal4 binding sites. At *GAL2*, a naturally DSB-cold locus, Gal4BD-Spo11 creates a recombinational hotspot that depends on all the other DSB gene functions, showing that the targeting of Spo11 to a specific site is sufficient to stimulate meiotic recombination that is under normal physiological control.

Introduction

In sexually reproducing organisms, the halving of the DNA content of a diploid germ line cell during the meiotic cell cycle allows for the production of haploid gametes. During this process, recombination plays a dual role: it shuffles information along the lengths of homologous chromosomes, creating the genetic diversity that is transmitted to progeny, and it ensures the proper segregation of homologs to opposite poles during the first of the two meiotic divisions (for review, Lee and Orr-Weaver, 2001). As engagingly reviewed by H.L.K. Whitehouse (1982), about a century ago de Vries (1903) predicted that exchanges take place between homologous maternal and paternal chromosomes during hereditary transmission. Soon thereafter, Bateson (1905) discovered partial linkage between the petal color and pollen shape characters in Sweet Pea. These and successive discoveries in the emerging field of recombination led to the notion of genetic distances, as measured by the frequency of exchange (crossing over) between linked markers, and to the development of linkage maps (Morgan and Cattell, 1912; Sturtevant, 1913). Sturtevant wrote, "Of course, there is no knowing whether or not these distances as drawn represent the actual relative spacial distances apart of the factors." Since the advent of the molecular era, this prescient insight has been extensively verified by quantitative comparisons of genetic and physical distances. For all organisms examined, including the yeast *Saccharomyces cerevisiae* (Goffeau et al., 1997), *Arabidopsis thaliana* (Mozo et al., 1999), *Drosophila melanogaster* (Noor et al., 2001), *Mus musculus* (Moore et al., 1999), and man (Yu et al., 2001), meiotic recombination rates (expressed as cM/kb) vary by almost two orders of magnitude along chromosomes, such that some loci are recombinational "hotspots" and others are "coldspots". In *S. cerevisiae*, meiotic recombination results from the formation and repair of programmed DNA double-strand breaks (DSBs) (for reviews: Lichten and Goldman, 1995; Smith and Nicolas, 1998; Zickler and Kleckner, 1998, 1999; Baudat and Keeney, 2001; Keeney, 2001; Petes, 2001). Numerous studies have shown that natural DSB sites are not evenly distributed and that cleavage frequencies vary 10–100-fold from site to site (Zenvirth et al., 1992; Wu and Lichten, 1994; Baudat and Nicolas, 1997; Gerton et al., 2000). The factors that determine whether a specific region or site is prone to DSB formation (and hence, recombination) are not completely understood, but they are known to act both locally and globally. Locally, gene organization and chromatin structure appear to be of paramount, and related, importance. Natural DSB sites are typically in promoter-containing regions (Wu and Lichten, 1994; Baudat and Nicolas, 1997). At the *HIS4* locus, two types of recombinational hotspot have been distinguished, α (transcription factor-dependent but not transcription-dependent) and β (transcription factor-independent) hotspots (Petes, 2001). More notably, all known DSB sites are located in regions that are sensitive to DNaseI or micrococcal nuclease (MNaseI) in both mitotic and meiotic cells, suggesting that an open chromatin configuration is necessary for cleavage (Ohta et al., 1994; Wu and Lichten, 1994; Fan and Petes, 1996; Keeney and Kleckner, 1996). However, local chromatin accessibility cannot be the sole arbiter of DSB site selectivity, because not all nuclease-hypersensitive sites are DSB sites.

Global determinants also control the distribution of DSBs. Both high resolution on yeast chromosome III and genome-wide mapping of DSB sites demonstrate

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that chromosomes can be subdivided into large domains hot or cold for DSB formation (Zenvirth et al., 1992; Baudat and Nicolas, 1997; Gerton et al., 2000). The molecular basis of these DSB-proficient or -refractory domains has not been elucidated, but the finding that a recombination-proficient reporter inserted at various sites along chromosome III adopts local properties with respect to DNaseI sensitivity and frequencies of DSB formation and recombination demonstrates that domain-level controls are superimposed on local determinants (Borde et al., 1999). Collectively, these studies indicate that hot regions can be made cold, but thus far the converse has not been observed: cold regions typically remain cold.

DSB frequency variation is also influenced by *trans*-acting factors. Numerous genes are essential for DSB formation, including *SPO11*, *MEI4*, *MER2/REC107*, *MRE2/NAM8*, *MRE11*, *RAD50*, *REC102*, *REC103/SKI8*, *REC104*, *REC114*, and *XRS2*, but in most cases, their molecular roles are unknown (Keeney, 2001). Null mutants for all of the above genes are recombinationally defective in meiosis and produce inviable spores. Four other meiosis-specific genes are required for full levels of DSBs: *MEK1/MRE4*, *RED1*, *HOP1*, and *MER1*. *SPO11* encodes a protein that shares sequence similarity with the smaller subunit (Top6A) of the type II topoisomerase of the archaebacterium *Sulfobolus shibatae* (Bergerat et al., 1997). Spo11 remains covalently linked to the 5′-strand termini of DSB fragments in mutants (e.g., *rad50S*) that are defective for the 5′ to 3′ nucleolytic processing of DSB ends that normally precedes repair (Keeney et al., 1997), indicating that it is the catalytic component of the meiotic DSB cleavage activity. These and other studies have demonstrated that Spo11 orthologs may be universally required for meiotic recombination, strongly suggesting that DSBs initiate meiotic recombination in all sexually reproducing eukaryotes (Keeney, 2001). Site-directed mutagenesis has been used to identify regions of Spo11 that contribute to strand cleavage and DNA binding, demonstrating the functional significance of structural motifs conserved throughout the Spo11/Top6A family (Bergerat et al., 1997; Diaz et al., 2002). Interestingly, variations in the level and distribution of DSBs at the *his4::LEU2* hotspot in some *spo11* mutants suggests that Spo11 is not only involved in cleavage but also participates in DSB site selection, at least locally (Diaz et al., 2002).

To test whether Spo11 participates in selecting DSB regions at the genome-wide level and to determine whether it could stimulate recombination in cold regions, we altered its substrate specificity by fusing it to the DNA binding domain of the Gal4 protein (Gal4BD), creating a Gal4BD-Spo11 chimeric protein. The Gal4 protein, one of the best characterized transcriptional activators in *S. cerevisiae*, binds to a consensus 17-base pair ($CGGN_{11}CCG$) upstream *a*ctivator *s*equence (UAS_{GAL}) through its N-terminal domain (Johnston, 1987). We report here that the Gal4BD-Spo11 fusion protein stimulates DSB formation with associated recombination near known UAS_{GAL} sites and Gal4 consensus binding sites, even in naturally cold DSB regions.

Results

The Gal4BD-Spo11 Fusion Protein Is Expressed in Meiosis

A fusion encoding the DNA binding domain of Gal4 (Gal4BD, amino acids 1–147) with the N terminus of the full-length *S. cerevisiae* Spo11 protein was placed under the control of the *ADH1* promoter (Figure 1A) and integrated at the *TRP1* locus in a *spo11*Δ strain. Homozygous diploids were derived by genetic crosses (Table 1). To verify expression, total RNA was analyzed during vegetative growth and in meiosis by Northern blot analysis. In wild-type diploids, *SPO11* mRNA begins to accumulate after transfer of cells to sporulation medium and decreases after 7 hr, by which time ascospores have begun to form (Figure 1B). In contrast, *GAL4BD-SPO11* mRNA is detected in both vegetative and meiotic cells, but like *SPO11* mRNA, it decreases at later time points (6–7 hr). Transcript quantification (Figure 1C) indicates that the fusion is highly expressed in vegetative cells and that the *GAL4BD-SPO11* mRNA levels are only slightly greater than *SPO11* mRNA levels during meiotic prophase (2–6 hr), most likely because the *ADH1* promoter is less active in meiotic than in mitotic cells (Chu et al., 1998), whereas the *SPO11* promoter is strongly induced (Atcheson et al., 1987). Finally, Western blot analysis shows that the fusion protein is expressed in both mitotic and meiotic *GAL4BD-SPO11* cells and is of the expected size, 63.5 kDa (Figure 1D).

Gal4BD-Spo11 Complements the *spo11*Δ Sporulation Defect

Diploid *spo11*Δ cells can sporulate but produce inviable progeny because chromosomes segregate abnormally when meiotic recombination is not initiated (Klapholz et al., 1985). We asked whether the Gal4BD-Spo11 protein could complement a *spo11*Δ diploid in this respect. Like wild-type *SPO11* (ORD5740) and *spo11*Δ (ORD5805) strains, a *GAL4BD-SPO11 spo11*Δ diploid (ORD5806) sporulates efficiently (approximately 80%), giving rise primarily to four-spored asci. Tetrad analysis shows that, as expected, none of the spores produced by *spo11*Δ diploids germinates (0/512). In contrast, spores derived from the *GAL4BD-SPO11 spo11*Δ diploid are fully viable (531/552, 96%), the same as observed for progeny of the *SPO11* diploid (505/526, 96%). High spore viability was similarly found for a diploid that contains both *GAL4BD-SPO11* and *SPO11* (ORD5817). Altogether, these results demonstrate that the *GAL4BD-SPO11* construct is functional and that it confers no adverse effect.

Gal4BD-Spo11 Promotes DSB Formation at Natural Sites

Since spore viability depends on meiotic recombination between each of a pair of homologous chromosomes, the complementation of the *spo11*Δ defect by *GAL4BD-SPO11* suggested that the fusion protein might also rescue the *spo11*Δ DSB defect. To test this idea, we examined several regions of the genome at which meiotic DSBs normally form. The *YCR043c-48w* region of chromosome III contains numerous DSB sites, including a strong hotspot in the *YCR047c-48w* intergenic region

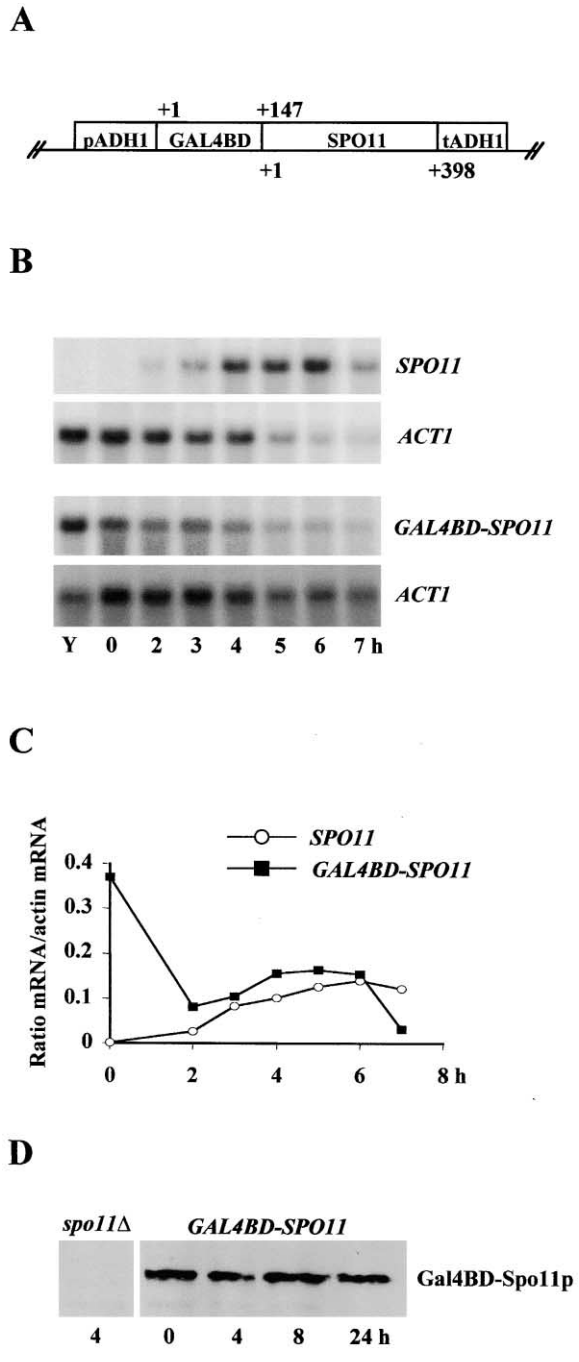


Figure 1. Structure and Expression of *GAL4BD-SPO11*

(A) The plasmid pAP1 containing the *GAL4BD-SPO11* fusion under the control of the *ADH1* promoter (pADH1) and terminator (tADH1) was constructed as described in Experimental Procedures. Amino acid residues derived from the Gal4BD and Spo11 proteins are indicated above and below, respectively.

(B) For Northern analysis, total RNA was prepared from *SPO11* (ORD5740) and *GAL4BD-SPO11* (ORD5806) cells during vegetative growth (Y) or at different times after transfer to sporulation medium and hybridized with a *SPO11* probe. Blots were rehybridized with an *ACT1* probe to provide a basis of comparison.

(C) Quantification of the levels of *SPO11* (circles) and *GAL4BD-SPO11* (squares) transcript with respect to the level of *ACT1* mRNA over the course of meiosis.

(D) The Gal4BD-Spo11 protein was detected by Western blot analysis using an anti-Gal4(DBD) antibody. Equivalent amounts of total protein are loaded on each lane, indicating that the fusion protein is present at a constant level in ORD5806 cells throughout meiosis. No signal was detected for *spo11Δ* diploids (ORD5805).

(Zenvirth et al., 1992; Baudat and Nicolas, 1997). As shown in Figure 2A, these DSBs form in both *SPO11* and *GAL4BD-SPO11* meiotic cells. In the *GAL4BD-SPO11* strain two DSB sites can be seen within the *YCR048w* coding region, which is notable since natural DSBs are generally restricted to promoter-containing regions (Figure 2A, and see below). A quantitative analysis of DSB band intensity indicates that the cumulative frequency of meiotic DSBs in this 9.6 kb chromosomal region is similar in both strains (approximately 13 ± 2%). In most cases, the DSB frequency at a given site is also similar, although DSBs form at a lower frequency in the *YCR047c-48w* intergenic region in the *GAL4BD-SPO11* diploid than in the *SPO11* diploid (5 ± 1% versus 11 ± 1%, respectively) (Figure 2B). Interestingly, the decrease in DSB frequency at this site is locally offset by the appearance of DSBs in the *YCR048w* ORF (4 ± 1%); this redistribution is suggestive of competition between adjacent DSB sites, as previously reported for Spo11-initiated DSBs (Wu and Lichten, 1995). We also examined two other well-characterized regions, the *ARG4* and *CYS3* loci (Sun et al., 1989; Vedel and Nicolas, 1999). In both cases, we detected similar meiotic DSB profiles in *SPO11* and *GAL4BD-SPO11* cells, with respect to positioning and intensity (Figures 2C and 2D). Altogether, these results demonstrate that the Gal4BD-Spo11 protein can promote DSB formation at natural sites at frequencies comparable to those promoted by wild-type Spo11, thereby explaining its ability to fully complement the inviability of *spo11Δ* spores.

Gal4BD-Spo11 Promotes DSB Formation near Gal4 Consensus Binding Sites

As described above, we observed two additional DSB sites in the *YCR048w* coding region (Figure 2A). Although these DSBs could result from an aberration in the targeting specificity of the Spo11 protein domain per se, at least in regions such as the *YCR048w* locus where Spo11 is already active, an examination of the *YCR048w* ORF revealed two internal Gal4 consensus binding site motifs at the estimated positions of the DSBs. This observation indicates that the Gal4BD-Spo11 protein can target DSBs to specific sequences, at least in this cleavage-proficient chromosomal domain (Baudat and Nicolas, 1997).

To determine whether the tethering of Gal4BD to Spo11 allows meiotic DSBs to be targeted to known Gal4 binding sites, we examined several loci containing UAS_{GAL} sites that have been implicated in galactose catabolism (Johnston, 1987). The *GAL2* promoter region contains four UAS_{GAL} sequences, two of which are constitutively bound by Gal4 in vivo (Huibregtse et al., 1993; Ren et al., 2000). In a *SPO11* strain, DSBs are barely detectable at the *GAL2* locus, indicating that this region is not a frequent natural DSB site. In contrast, in a *GAL4BD-SPO11* strain, prominent DSBs can be observed in the *GAL2* promoter near or at the UAS_{GAL} sites

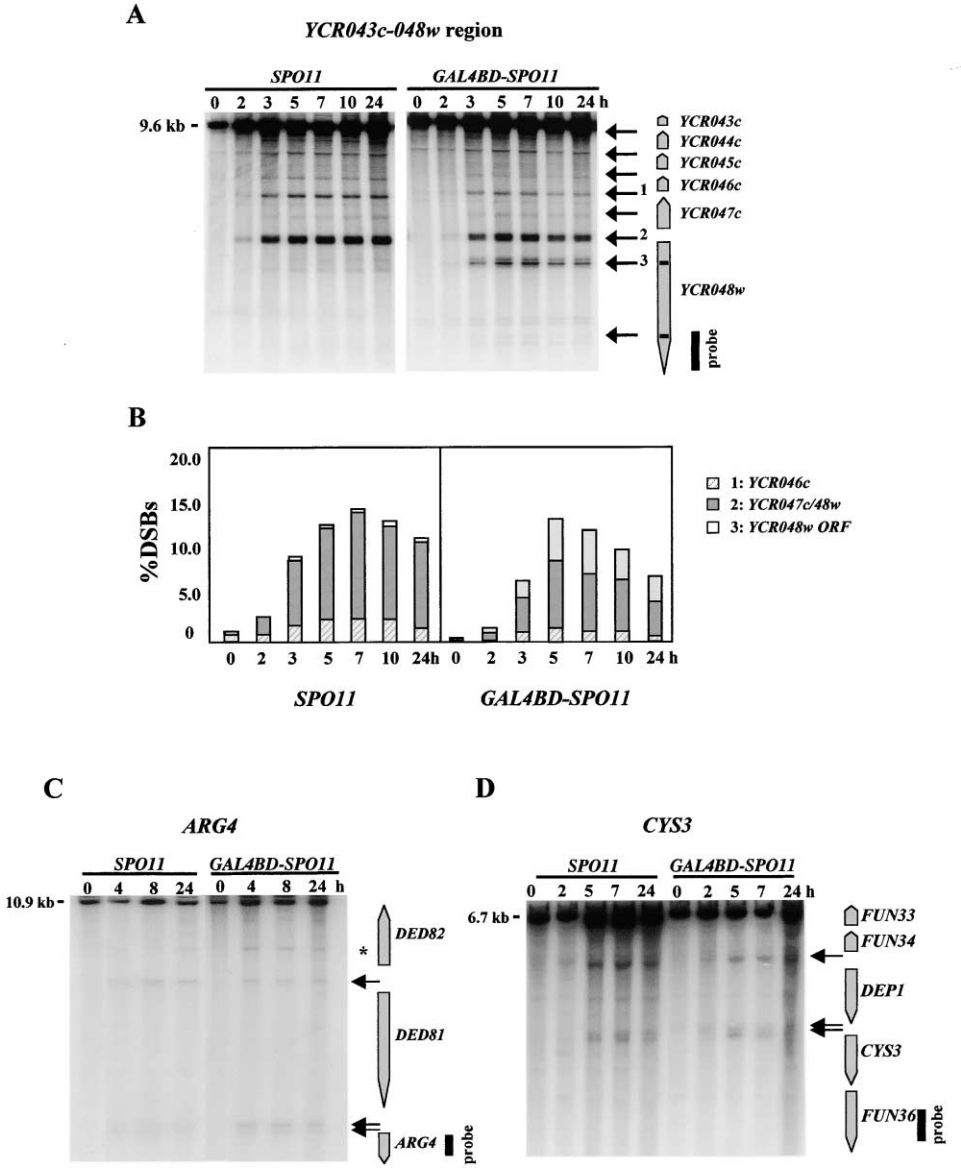


Figure 2. Meiotic DSB Formation at the Natural *YCR043c-YCR048w*, *ARG4*, and *CYS3* Hotspots in *SPO11* and *GAL4BD-SPO11* Diploids

Genomic DNA was prepared from *SPO11* (ORD1181) and *GAL4BD-SPO11* (ORD5807) diploids taken at the indicated time after transfer to sporulation medium and DSBs were detected by Southern analysis. These strains are homozygous for the *rad50S::URA3* allele, which permits DSB formation but prevents resection and repair (Alani et al., 1990). At the right of each gel, a map of the region shows ORFs (open arrows indicate transcriptional sense), DSB sites (arrows), and the positions of the probes.

(A) DSB formation in the *YCR043c-48w* region. DNA was digested with AseI and probed with a *YCR048w* internal fragment. Gal4 consensus binding sequences in the *YCR048w* ORF are shown as black bars.

(B) Quantification of prominent DSBs in the *SPO11* and *GAL4BD-SPO11* cells shown in Figure 2A. The sums of the frequencies of DSBs in the *YCR046c* promoter (1), the *YCR047c-48w* promoter (2), and the *YCR048w* ORF (3) are represented as a histogram. These sums account for $\geq$ 99% of the total DSBs detected in the *YCR043c-48w* interval.

(C) DSB formation at the *ARG4* locus. DNA was digested with SnaBI and probed with an *ARG4* internal fragment. The asterisk indicates a crosshybridizing band.

(D) DSB formation at the *CYS3* locus. DNA was digested with HindIII and probed with a *FUN36* internal fragment.

(Figure 3A). Similar to what was observed for the *YCR043c-48w* interval, DSBs appear at the *GAL2* locus in *GAL4BD-SPO11* meiotic cells within 2 hr of transfer of cells to sporulation medium, and their intensity increases over the course of meiosis, reaching a maximum at 8–10 hr. Quantitative analysis of several independent experiments indicates that the frequency of *GAL2* DSBs in the *GAL4BD-SPO11* strain is approximately 12 $\pm$ 2%, in contrast to $\leq$ 0.6% observed in the wild-type strain, a 20-fold stimulation (Figure 3A).

To generalize our observations, we examined the *GAL7*, *GAL10*, and *GAL1* genes, which like *GAL2* are

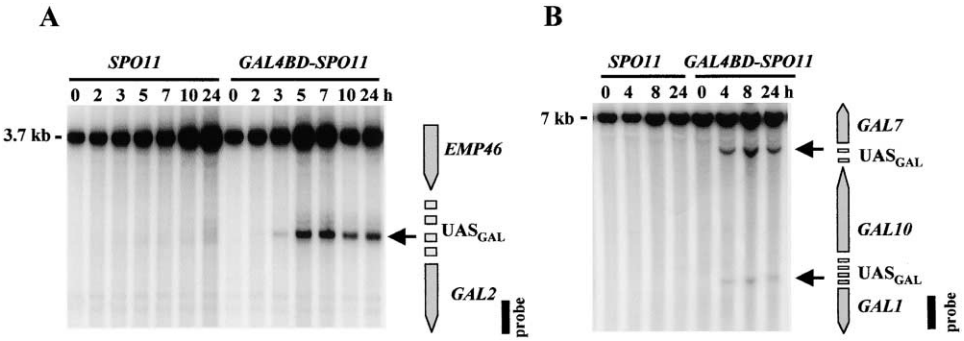


Figure 3. *GAL4BD-SPO11*–Promoted DSBs at Loci with UAS$_{GAL}$ Sequences

Genomic DNA was prepared from *SPO11* (ORD1181) and *GAL4BD-SPO11* (ORD5807) diploids and analyzed as described in Figure 2. UAS$_{GAL}$ sequences are indicated as boxes.

(A) DSB formation at the *GAL2* locus. DNA was digested with XbaI and NcoI and probed with a *GAL2* internal fragment.

(B) DSB formation at the *GAL1,7,10* locus. DNA was digested with ClaI and AatII and probed with a *GAL1* internal fragment.

transcriptionally induced by Gal4 in the presence of galactose. At these loci, meiotic DSBs do not form in *SPO11* diploids but in contrast are readily detectable at or near their associated UAS$_{GAL}$ sites in *GAL4BD-SPO11* diploids (Figure 3B). Quantitative analysis indicates a substantial stimulation of DSB formation, with frequencies of 4 ± 1% for the *GAL7* upstream region and 2 ± 1% for the divergently transcribed *GAL1-GAL10* intergenic region. This is notably lower than the frequency of 12 ± 2% observed for the *GAL2* promoter (Figure 3A). Altogether, we conclude that the Gal4BD-Spo11 protein can target meiosis-specific DSBs to Gal4 DNA binding sites, creating novel genomic DSB sites of varying strength.

Finally, we verified with several control strains that the Gal4BD-Spo11 fusion protein is solely responsible for the appearance of these DSBs (data not shown). First, *spo11Δ* strains expressing the Gal4BD-spo11Y135F fusion protein, in which the presumed catalytically active tyrosine residue of Spo11 is replaced by phenylalanine (Bergerat et al., 1997), do not exhibit meiotic DSBs either in the *YCR043c-48w* region or at the *GAL2* locus. Second, *SPO11* strains expressing the Gal4 binding domain either alone or fused to Rpb5 (an RNA polymerase subunit whose function is unrelated to the initiation of recombination) and a strain expressing a *pADH1::SPO11* construct (without the Gal4BD) exhibit DSBs in the *YCR043c-048w* region but not at the *GAL2* locus. Collectively, these control experiments demonstrate that the stimulation of Gal4BD-Spo11-dependent DSBs near Gal4 binding sites requires both the Gal4BD and Spo11 components of the chimeric protein.

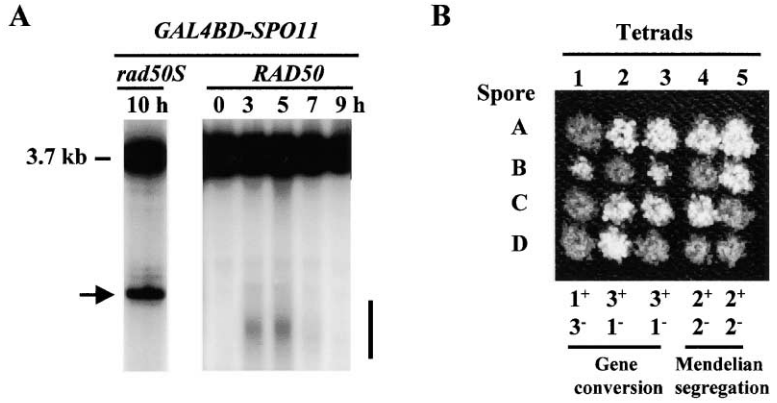
The Gal4BD-Spo11 Fusion Strongly Stimulates Recombination at the *GAL2* Locus

To determine whether the *GAL4BD-SPO11*-promoted DSBs are recombinogenic, we first examined meiotic DSB fragments in the *GAL2* region in a *RAD50* background to see if they are processed like Spo11-induced DSBs. We detected DSBs (Figure 4A) as a smear of fragments of greater mobility than the discrete *rad50S* band, showing that the *GAL2* DSBs undergo processing. Moreover, the transient nature of the smear (appearing by 3 hr and disappearing between 7–9 hr) suggests that these DSBs are repaired with normal kinetics.

To assess whether Gal4BD-Spo11-dependent DSBs are repaired by homologous recombination, we then measured the frequency of meiotic gene conversion at the *GAL2* locus. For this purpose, we constructed a mutant allele, *gal2-Bsp,* which confers a slow growth phenotype on galactose, and derived *GAL2/gal2-Bsp* heterozygotes in both the *SPO11* and *GAL4BD-SPO11* backgrounds. From 218 four-spore tetrads produced by the *SPO11* diploid, we found only five conversion events. In contrast, for the *GAL4BD-SPO11* strain, we observed 55 conversion events, as indicated by equivalent numbers of 3:1 and 1:3 tetrads, among 212 tetrads analyzed (26%), a 10-fold increase over the level observed for the *SPO11* strain (Figures 4B and 4C). Tetrads exhibiting a 4:0 or 0:4 configuration were not seen. Gal4BD-Spo11-promoted DSBs can also initiate recombination at other loci; we confirmed by tetrad analysis that the frequencies of meiotic gene conversion at the *ARG4* natural hotspot are similar in *SPO11* and *GAL4BD-SPO11* diploids (data not shown). These results show that the DSBs formed in the *GAL2* promoter in *GAL4BD-SPO11* strains are recombinogenic and behave like Spo11-induced breaks.

Gal4BD-Spo11 Promotes Cleavage in a Large Region that Is Normally Cold for DSB Formation

We extended our analysis of Gal4BD-Spo11 activity to an approximately 20 kb interval centered on *GAL2* (between the *YLR072w* and *RAX2* ORFs) that contains eight intergenic promoter-containing regions (Figure 5A). In a *SPO11* strain, we observed no DSBs in this interval. In a *GAL4BD-SPO11* strain, the *GAL2* promoter region is the only site at which DSBs can be detected. Since there are no other Gal4 consensus sequences in this interval, these results underscore three important features of Gal4BD-Spo11 DSB activity. First, the stimulation of DSBs near *GAL2* is specifically targeted. Second, cleavage at *GAL2* does not promote the nearby formation of other DSBs. Third, in contrast to what is observed for



C

	GAL2/gal2-Bsp segregation			
	2+ 2-	3+ 1-	1+ 3-	Gene conversion
SPO11	213	2	3	2%
GAL4BD-SPO11	157	28	27	26%

Figure 4. Recombination Is Stimulated at the *GAL2* Locus in a *GAL4BD-SPO11* Strain

(A) DSB formation at the *GAL2* locus in a *RAD50* strain (ORD5806). Genomic DNA was digested with XbaI-NcoI and probed with a *GAL2* internal fragment. Arrow at left shows DSBs at the *GAL2* UAS_{GAL} site in an isogenic *rad50S* strain (ORD 5807); the bar at right indicates the extent of the "smear" of DSB fragments.

(B) Segregation of the *GAL2* and *gal2-Bsp* alleles among progeny of a *GAL4BD-SPO11* diploid (ORD6626). Tetrads were dissected on YPD and colonies were replica plated to YPGal. The *gal2-Bsp* allele confers a slow growth phenotype. Gene conversions (3+:1− and 1+:3−) and Mendelian (2+:2−) segregation patterns can be seen in this example.

(C) Meiotic gene conversion frequencies at the *GAL2* locus in *GAL4BD-SPO11* (ORD6626) and *SPO11* (ORD6632) diploids heterozygous for the *GAL2* and *gal2-Bsp* alleles.

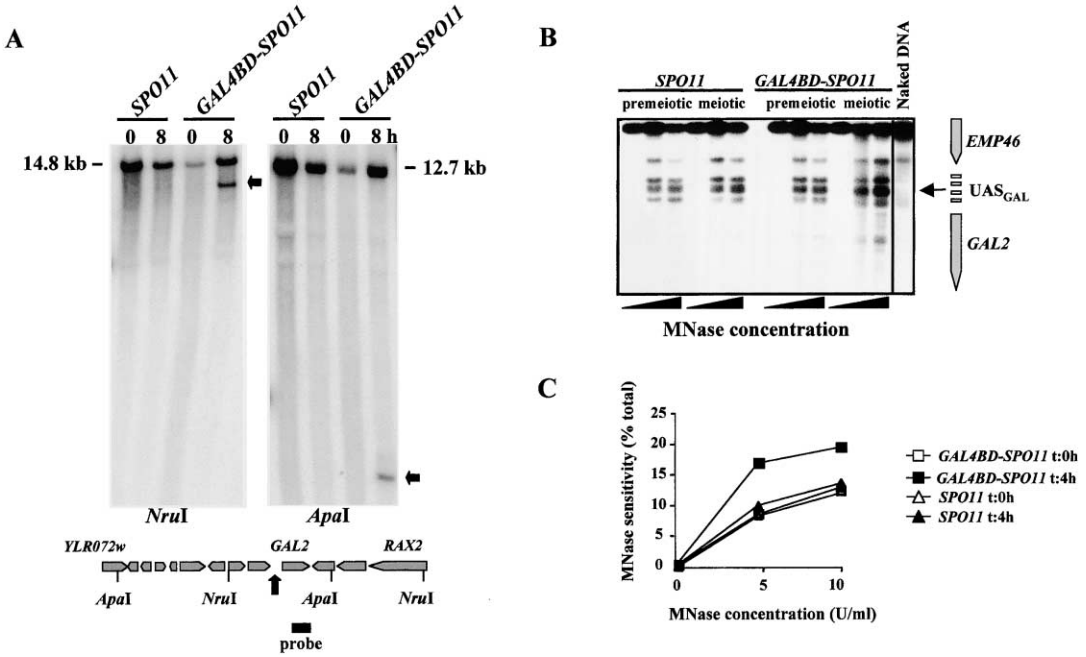


Figure 5. The *GAL2* Locus Is Located in a Normally Cold Region but DSBs Promoted by the Gal4BD-Spo11 Protein Are Associated with Changes in Chromatin Accessibility

(A) Southern blot analysis of DSB formation in a 20 kb interval centered on the *GAL2* locus. Meiotic DNA from *SPO11* (ORD1181) and *GAL4BD-SPO11* (ORD5807) diploids was digested with the indicated restriction enzymes and probed with a *GAL2* internal fragment. The schematic shows the transcriptional senses of genes and relevant restriction sites. Arrows indicate DSB sites.

(B) The meiotic chromatin transition at the *GAL2* locus. Chromatin was isolated from *SPO11* (ORD5740) and *GAL4BD-SPO11* (ORD5806) cells in presporulation medium (premeiotic) or from cells after 4 hr in sporulation medium (meiotic), treated with MNase (0, 5, 10, or 20 U/ml, as represented by solid triangles), and analyzed by an indirect end-labeling method. The diagram at right shows the position of the *GAL2* ORF in the parental fragment. The arrow indicates the *GAL2* MNase-hypersensitive site at the UAS_{GAL} sequences (boxes).

(C) Quantification of MNase sensitivity at the Gal4 binding site of *GAL2*, expressed as the percentage of radioactivity for the band indicated by an arrow in (B) relative to total lane radioactivity and plotted as a function of MNase concentration.

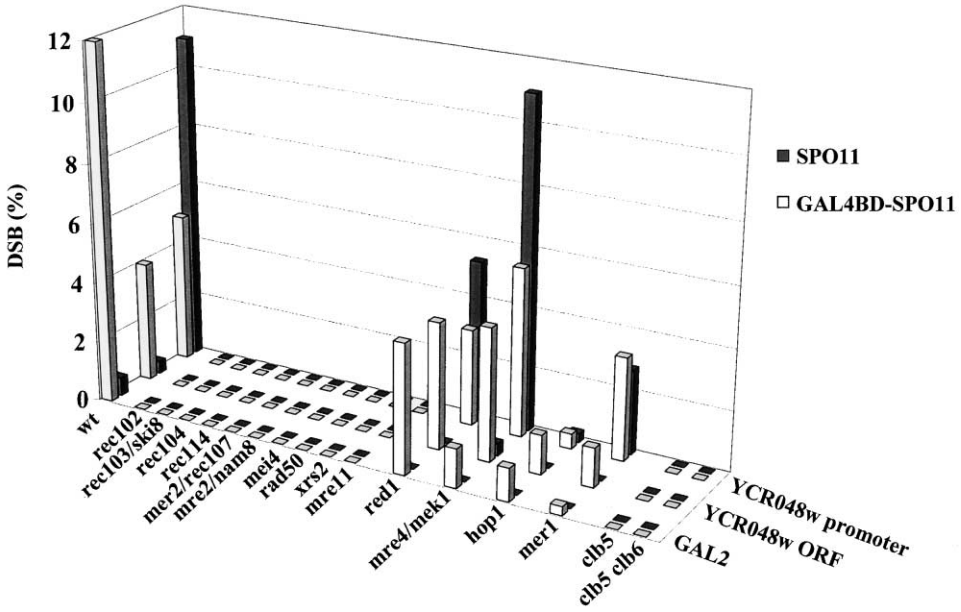


Figure 6. Genetic Requirements for DSB Formation in *SPO11* and *GAL4BD-SPO11* Strains

Southern blot analyses were carried out as in Figures 2 and 3 to measure meiotic DSB frequencies (*z* axis) at the *GAL2* locus, *YCR048w* ORF and *YCR048w* promoter (*y* axis) in *rad50S* (or *com1*Δ, in the case of *rad50*Δ and *xrs2*Δ) diploids carrying the *SPO11* or *GAL4BD-SPO11* constructs (black or white bars, respectively), in wild-type and mutant (*x* axis) backgrounds. Strain genotypes are listed in Table 1. DSB frequencies were determined at 8 or 10 hr after transfer to sporulation medium.

DSBs in the *YCR048w* region of chromosome III, the *GAL2* DSBs occur in a naturally cold chromosomal region.

DSBs at *GAL2* Are Accompanied by a Local Increase in MNase Sensitivity during Early Meiosis

The hypersensitivity of chromatin to MNase at hotspots increases during early meiotic prophase, before DSB formation (Ohta et al., 1994). To determine whether the presence of Gal4BD-Spo11 alters the accessibility of chromatin to nucleases at future DSB sites, we assessed the effects of MNase on the *GAL2* locus during meiosis (Figure 5B). We found no significant meiotic change in MNase sensitivity at this locus in *SPO11* diploids, consistent with the absence of DSBs. In contrast, *GAL4BD-SPO11* cells exhibit a meiosis-specific increase in sensitivity, as seen after 4 hr in sporulation medium (Figures 5B and 5C), a time at which DSBs are forming. In strains expressing Gal4 either alone or fused to Rpb5, we observed that the chromatin configuration of the *GAL2* locus is unaffected (data not shown). Thus, the meiosis-specific chromatin transition observed at the *GAL2* locus requires the presence of the Gal4BD-Spo11 construct and is not simply due to an enhanced binding of the Gal4 DNA binding domain to target sequences in the *GAL2* promoter.

DSB Formation by Gal4BD-Spo11 Depends on the Other Known DSB Genes

We reasoned that if one or more of the other proteins involved in DSB formation is necessary to recruit Spo11 to future DSB sites, the addition of the Gal4 DNA binding domain to Spo11 might bypass this requirement, at least for DSBs at Gal4 binding sites. Southern blot analysis indicates that *rec102*, *rec103/ski8*, *rec104*, *rec114*, *mei4*, *mer2/rec107*, *mre2/nam8*, *mre11*, *rad50*, and *xrs2* null mutants, in either the *SPO11* or *GAL4BD-SPO11* background, do not exhibit meiotic DSBs in the *YCR043c-048w* interval or at *GAL2* (Figure 6). The frequency of Gal4BD-Spo11 promoted DSBs at the *GAL2* locus is reduced about 3-fold in *red1* mutants and 10-fold in *mre4/mek1* and *hop1* mutants; DSBs are almost undetectable in *mer1* mutants. In the *YCR048w* region (promoter + ORF), the *red1*, *mer1*, and *hop1* mutations have increasingly repressive effects in both strains but the *mre4/mek1* mutation has no effect. The differential effects of each of these mutations on chromosome III and at *GAL2* may reflect locus-specific variation, but overall, these results show that DSB formation in *GAL4BD-SPO11* strains depends on the same proteins that are important for cleavage in *SPO11* strains.

Gal4BD-Spo11 Cleavage also Requires Clb5 Clb6 Activity

The B-type cyclins *CLB5* and *CLB6* are required for meiotic replication (Chu et al., 1998; Dirick et al., 1998; Stuart and Wittenberg, 1998) and for meiotic DSB formation (Smith et al., 2001). To determine whether Gal4BD-Spo11-promoted DSBs also depend on Clb5 and Clb6 activity, we assayed DSB formation in *clb5* and *clb5 clb6* diploids containing the *GAL4BD-SPO11* construct (Table 1). As indicated in Figure 6, we did not detect DSBs at either the *YCR048w* or the *GAL2* locus in these strains, demonstrating that the addition of a heterolo-

Table 1. Strain Genotypes

Strain	Relevant Genotype
ORD1181	*a/α rad50S::URA3/"*
ORD5740	*a/α arg4-nsp/arg4-bg LEU2/leu2*
ORD5805	*a/α spo11Δ::URA3/" arg4-bg/arg4-rV NUC1/nuc1Δ::LEU2 leu2/"*
ORD5806	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" arg4-bg/arg4-rV nuc1Δ::LEU2/" leu2/"*
ORD5807	*a/α trp1::G4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rad50S::URA3/" arg4-bg/" NUC1/nuc1Δ::LEU2 leu2/"*
ORD5817	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" arg4-bg/ARG4 leu2/"*
ORD5821	*a/α rec102Δ::URA3/" rad50S::LEU2/" leu2/"*
ORD5828	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mre4::URA3/" rad50S::LEU2/" arg4-bg/arg4-rV leu2/"*
ORD5845	*a/α mre4::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD5846	*a/α mre2::URA3/" rad50S::LEU2/" leu2/"*
ORD5849	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mre2::URA3/" rad50S::LEU2/" arg4-rV/" leu2/"*
ORD5851	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rec104Δ::LEU2/" rad50S::URA3/" arg4-bg/" leu2/"*
ORD5857	*a/α rec104Δ::LEU2/" rad50S::URA3/" leu2/"*
ORD5859	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rec102Δ::URA3/" rad50S::LEU2/" arg4-rV/ARG4 leu2/"*
ORD5879	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rec103::KanMX/" rad50S::URA3/" arg4-bg/ARG4 leu2/"*
ORD6534	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" clb5::URA3/" clb6::TRP1/" rad50S::LEU2/" arg4-bg/" leu2/"*
ORD6551	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" clb5::URA3/" rad50S::LEU2/" arg4-bg/" leu2/"*
ORD6591	*a/α mei4::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD6593	*a/α rec103::KanMX/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD6595	*a/α red1::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD6598	*a/α rec114::KanMX" rad50S::LEU2/" arg4-bg/" leu2/"*
ORD6626	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/"arg4-bg/ARG4 GAL2/gal2-Bsp NUC1/nuc1Δ::LEU2 leu2/"*
ORD6632	*a/α GAL2/gal2-Bsp LEU2/leu2*
ORD7414	*a/α mer1::LEU2/" rad50S::URA3/" arg4-rV/" leu2/"*
ORD7419	*a/α hop1::LEU2/" rad50S::URA3/" arg4-rV/" leu2/"*
ORD7420	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mei4::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD7421	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rec114::KanMX/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD7430	*a/α mre11Δ::KanMX/" rad50S::URA3/" arg-rV/ARG4 leu2/"*
ORD7443	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mer1::LEU2/" rad50S::URA3/" arg-rV/ARG4 leu2/"*
ORD7447	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" red1::URA3/" rad50S::LEU2/" arg4-bg/" leu2/"*
ORD7448	*a/α mer2::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD7452	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" rad50Δ::hisG/" com1::KanMX/" arg4-nsp/ARG4 leu2/"*
ORD7454	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" hop1::LEU2/" rad50S::URA3/" arg4-rV/ARG4 leu2/"*
ORD7456	*a/α rad50Δ::hisG/" com1::KanMX/" arg4-nsp, bg/ARG4 LEU2/leu2*
ORD7459	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mer2::URA3/" rad50S::LEU2/" arg4-bg/ARG4 leu2/"*
ORD7461	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" mre11Δ::KanMX/" rad50S::LEU2/" arg4-bg/" leu2/"*
ORD7466	*a/α xrs2Δ::LEU2/" com1::KanMX/" arg4-nsp,bg/ARG4 leu2/"*
ORD7468	*a/α trp1::GAL4BD-SPO11-TRP1-KanMX/" spo11Δ::URA3/" xrs2Δ::LEU2/" com1:: KanMX /" arg4-nsp,bg/ARG4 leu2/"*

All strains are *ho::LYS2/" lys2/" ura3/" trp1/"*.
All strains were constructed for this work except ORD1181 (Baudat and Nicolas, 1997) and ORD5740 (Smith et al., 2001).

gous DNA binding domain to Spo11 does not bypass the requirements for Clb5 and Clb6 activity and hence does not overcome the meiotic replication defect that is relevant to DSB induction. In conjunction with the demonstrated requirement for the other DSB genes, this result indicates that the Gal4DB-Spo11 protein is subject to the same (*trans*-acting) genetic controls as the Spo11 protein for DSB formation at both natural and targeted sites.

Discussion

The results described here show that the Gal4BD-Spo11 fusion acts like a wild-type Spo11 protein in promoting DSB formation at natural sites and that it also stimulates targeted DSB formation and recombination near known Gal4 binding sites, even in naturally cold regions. Moreover, our observations that a meiosis-specific chromatin transition takes place at the targeted *GAL2* locus in *GAL4BD-SPO11* strains and that the other known DSB genes are required or important for cleavage have implications for defining a pre-DSB scenario.

The Gal4BD-Spo11 Protein Retains the Basic Activity of Spo11

The Gal4BD-Spo11 protein functionally resembles the wild-type Spo11 protein in many ways. First, it catalyzes meiotic DSB formation at known hotspot loci. Except in the *YCR048w* ORF, where we detected competition between natural and novel sites (see below), DSB frequencies were similar in all regions examined in *GAL4BD-SPO11* and *SPO11* diploids. Second, in the *RAD50* background the DSBs appear, undergo resectioning, and disappear with the kinetic profile of DSBs in wild-type strains, indicating that they are formed and processed normally. Third, these targeted DSBs promote 3:1 and 1:3 gene conversion events, indicating that recombination can be initiated on either homolog and that only one of a pair of sister chromatids is cleaved. Fourth, the absolute level of meiotic gene conversion involving the *gal2-Bsp* marker (in 26% of cells undergoing meiosis) is consistent with the frequency of DSBs in the promoter region, indicating that as at other meiotic hotspots, these DSBs are preferentially repaired using a homologous chromosome rather than a sister chromatid as a template. Finally, the fusion protein fully comple-

ments a *spo11* deletion in terms of spore viability, suggesting that genome-wide levels of recombination associated with crossing over are sufficient to ensure proper chromosome segregation. We conclude that the addition of the 147 amino acid Gal4 binding domain to the N terminus of the Spo11 protein does not prevent Spo11 from binding to its natural targets, from interacting with other proteins that may be involved in forming a functional DSB complex, and, ultimately, from cleaving DNA. This may not be surprising since the Gal4 DNA binding domain has been added to other DNA binding proteins (Fok1, Topo1) without interfering with their activities (Kim et al., 1998; Beretta et al., 1999).

The Gal4BD-Spo11 Protein Targets Meiotic DSBs to Gal4 Binding Sites, even in Naturally Cold Regions

In addition to the normal features of Spo11, the Gal4BD-Spo11 protein has a property: it targets meiotic DSBs to regions containing well-characterized UAS_{GAL} sequences (*GAL2*, *GAL7*, and *GAL1/10*) (Johnston, 1987). The absolute frequency of DSB formation in these regions varies: it is high (12%) at *GAL2*, comparable to what is observed at the most active hotspots in the yeast genome (Gerton et al., 2000), and it is lower at *GAL7* and *GAL1/GAL10* (4% and 2%, respectively). This locus-to-locus variation does not directly correlate with the number of consensus Gal4 binding sequences in the *GAL2*, *GAL7*, and *GAL1/10* promoters (4, 2, and 4, respectively) nor with the binding affinities of Gal4 to these regions (Ren et al., 2000). Although these parameters do not necessarily predict how the Gal4 binding domain might behave during meiosis, the observation that Gal4 constitutively binds its target sites even in the absence of galactose (Huibregtse et al., 1993) suggests that the variation of *GAL4BD-SPO11*-specific DSB frequencies reflects other controls. This is also the case for wild-type meiosis, in which the frequencies of DSBs in proficient domains are highly variable.

We also observed a significant stimulation of DSB formation in the *YCR048w* ORF (Figure 2). DSBs rarely occur in ORFs in wild-type meiotic cells (Baudat and Nicolas, 1997), with the exception of a few loci such as *HIS2* in *S. cerevisiae* (Bullard et al., 1996) and the *ade6-M26* allele of *Schizosaccharomyces pombe* (Steiner et al., 2002). The low but detectable levels of DSBs near the two Gal4 consensus binding sites in the *YCR048w* coding region strongly suggest that the Gal4BD-Spo11 protein can bind to Gal4 consensus sequences that are unlikely to play a role in transcriptional activation, thereby extending the spectrum of potential target sites throughout the genome to include intragenic regions.

Finally, the noteworthy absence of DSBs in a 20 kb interval centered on the *GAL2* locus in wild-type cells (Figure 5) and of detectable DSB hotspots in a larger interval (about 80 kb) containing *GAL2* (Gerton et al., 2000) indicate that the *GAL2* locus is not an isolated cold site in an otherwise hot chromosomal domain but is instead imbedded in a cold domain. Considering the strong stimulation of DSB formation at *GAL2* (at least 20-fold) by the Gal4BD-Spo11 protein, we conclude that the Gal4 DNA binding domain permits Spo11 to gain access to a cold region. In addition, these results suggest that the targeting of Spo11 to a naturally cold region is sufficient to attract all the other factors required to trigger DNA cleavage. Further, the stimulation of recombination at *GAL2* also indicates that the machinery necessary to repair these meiotic DSBs can be recruited to naturally cold regions.

The Steps Leading to DSB Formation: a Pre-DSB Scenario

The relative timing at which the various components of the DSB machinery come into play and how they interact with one another are unsettled issues. In particular, the way that Spo11 or the pre-DSB complex is attracted to future cleavage sites is unknown. It has been shown that there is a temporal and regional correlation between replication and DSB formation (Borde et al., 2000), and that the *spo11* deletion, but not the catalytically inactive spo11-Y135F protein, reduces the duration of meiotic S-phase (Cha et al., 2000). Considering these observations, a plausible hypothesis is that the progression of replication creates a DNA or chromatin substrate that allows Spo11 to be directly or indirectly recruited to future DSB sites. Thus, in an extension of this hypothesis, it is possible that besides its known role in cleavage and proposed function in refining the choice of DSB sites within a delineated region (Diaz et al., 2002), Spo11 could play an early and primary role in selecting future DSB regions, perhaps by interacting with as yet unidentified replication intermediates. Our first and most striking observation is that targeting Spo11 (through the Gal4BD motif) to a novel site promotes DSB formation, indicating either that Spo11 alone is sufficient to recruit all the other necessary DSB proteins or that a preexisting Spo11-containing complex, once targeted to a specific site, suffices to finally trigger cleavage. Of course, at natural sites, a member of the pre-DSB complex other than Spo11 may be responsible for cleavage site selection, but the present data are also compatible with the idea that Spo11 can act autonomously in this respect. A second significant finding concerns the increase in MNase sensitivity observed at *GAL2*, which is known to occur at Spo11-dependent natural DSB sites (Ohta et al., 1994, 1999). Increases in MNase sensitivity have been interpreted as either programmed modifications that subsequently allow Spo11 and other DSB proteins access to cleavage sites, or alterations induced by the dynamic assembly of DSB complexes at future DSB sites. The meiotic chromatin transition seen at *GAL2* in *GAL4BD-SPO11* cells and the additional observation that it does not occur in *spo11*Δ cells (M. Furuse and K. Ohta, unpublished data) strongly favor the latter hypothesis and suggest that the binding of Spo11 or a Spo11-containing protein complex to target sites directly or indirectly promotes the chromatin transition. Finally, our observation that like Spo11, the Gal4BD-Spo11 protein cannot introduce DSBs in the *clb5 clb6* double mutant, which is defective in premeiotic replication (Dirick et al., 1998; Stuart and Wittenberg, 1998) and in the chromatin transition (Smith et al., 2001), reinforces the growing evidence that Spo11 can only cleave DNA that is replicated in the meiotic context and provides a mechanistic link between meiotic replication and the initiation of recombination (Baudat and Keeney, 2001; Forsburg, 2002). Thus,

the differential recruitment, assembly, and/or maturation of Spo11 or Spo11-containing pre-DSB complexes along chromosomes provides a plausible explanation for the non-random distribution of natural DSBs sites, and hence recombination rates.

A Novel Method to Stimulate Germ Line Recombination

The results reported here provide the first evidence that the frequency of homologous recombination during meiosis can be substantially augmented at a targeted site by a simple modification of the Spo11 protein. Previous reports have described the use of site-specific nucleases such as I-SceI, HO, or VDE to locally stimulate meiotic homologous recombination at loci in which the targets of these enzymes have been introduced (Jasin, 1996; Malkova et al., 2000; Neale et al., 2002). Also, in at least two other cases, Spo11-dependent DSBs have been shown to form at novel locations: in *S. cerevisiae*, near telomeric sequences introduced upstream of *HIS4* (Xu and Petes, 1996) and in *S. pombe*, near sites of introduction of the M26 heptamer sequence (Steiner et al., 2002). The present method of adding a heterologous DNA binding domain to Spo11 provides an alternative strategy with distinct features and practical advantages. For example, this approach exploits naturally occurring chromosomal sites of low complexity, thereby multiplying the number of potential targets without requiring the prior introduction of a specific sequence or deletion of the endogenous *SPO11* gene. Also, cleavage remains under normal physiological control with respect to *cis*- and *trans*-acting determinants, and as a consequence, DSBs are repaired by the homologous recombination pathway, allowing for the production of viable gametes and the recovery of novel recombinants.

Experimental Procedures

Plasmid Construction

To create the integrative plasmid pAP1, which encodes the Gal4BD-Spo11 fusion, the *SPO11* ORF was generated by PCR and introduced as a BamHI/PstI fragment downstream of the sequence encoding the Gal4 DNA binding domain (Gal4BD) in the two-hybrid vector pAS2ΔΔ (a pAS2-1 derivative, Clontech), provided by P. Legrain. The in-frame N-terminal fusion of Gal4BD to Spo11 and the complete ORF were verified by sequencing. The *kan*MX4 drug resistance cassette (Wach, 1996) was inserted into an NruI site, and the 2μ replication origin was removed by replacing a BpmI-Bsu36I fragment with the corresponding fragment from pRS304 (Sikorski and Hieter, 1989), producing pAP1. To create pICM99, the *GAL2* locus was amplified from genomic DNA and a subclone containing the promoter region and the first 655 bp of the *GAL2* ORF was mutagenized by the addition of an adenosine nucleotide at position +197 with the QuikChange site-directed mutagenesis kit (Stratagene). This frameshift mutation generates a BspHI site and gives rise to a truncated protein of 66 amino acids. The relevant fragment was sequenced to confirm the mutation and finally subcloned into pRS306 (Sikorski and Hieter, 1989), producing pICM99. Details of plasmid constructions and primer sequences are available on request.

Yeast Strains

All yeast strains are isogenic derivatives of SK1 (Kane and Roth, 1974) and were obtained by transformation or crossing (details available upon request); their relevant genotypes are shown in Table 1. pAP1 was integrated at the *trp1* locus by transformation (Ausubel et al., 1988) with selection for G418 (200 μg/ml) resistance. The *gal2*-BspHI mutation was introduced at the *GAL2* locus by the two-step replacement procedure (Ausubel et al., 1988) using pICM99, which contains the *URA3* selectable marker. Constructs were verified by PCR and Southern analysis.

Media and Genetic Techniques

Standard rich media (YPD, YPGal) and SD (0.67% yeast nitrogen base without amino acids, 2% glucose), supplemented when necessary with appropriate nutrients (Ausubel et al., 1988), were used for vegetative growth. Meiotic cultures were prepared as described (Alani et al., 1990).

Molecular Techniques

For detection and quantification of DSBs, genomic DNA was prepared from sporulating cells and subjected to Southern analysis. Parental and DSB bands were visualized with a Phosphorimager and quantified with the use of ImageQuant software (Molecular Dynamics) as described (Vedel and Nicolas, 1999). Northern analysis was performed as described (Smith et al., 2001). Cell extracts were prepared and analyzed by Western blotting with an anti-Gal4(DBD) antibody (Santa Cruz Biotechnology) using standard procedures (Ausubel et al., 1988).

Analysis of Chromatin Hypersensitivity

Chromatin was isolated from premeiotic and meiotic cells and digested with micrococcal nuclease (Mnase; Ohta et al., 1994). After MNase treatment, DNA was digested with NcoI and XbaI and analyzed by Southern hybridization using a *GAL2* probe. Fragments representing hypersensitive sites were visualized with a Fuji-BAS2000 image analyzer and quantified as described (Ohta et al., 1994).

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References

Alani, E., Padmore, R., and Kleckner, N. (1990). Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell *61*, 419–436.

Atcheson, C.L., DiDomenico, B., Frackman, S., Esposito, R.E., and Elder, R.T. (1987). Isolation, DNA sequence, and regulation of a meiosis-specific eukaryotic recombination gene. Proc. Natl. Acad. Sci. USA *84*, 8035–8039.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Strul, K. eds. (1988). Current Protocols in Molecular Biology (New York: John Wiley and Sons, Inc.).

Bateson, W., Saunders, E.R., and Punnett, R.C. (1905). Experimental studies in the physiology of heredity. Rep. Evol. Comm. R. Soc. *2*, 1–55, 80–99.

Baudat, F., and Nicolas, A. (1997). Clustering of meiotic double-

strand breaks on yeast chromosome *III*. Proc. Natl. Acad. Sci. USA *94*, 5213–5218.

Baudat, F., and Keeney, S. (2001). Meiotic recombination: making and breaking go hand in hand. Curr. Biol. *11*, R45–R48.

Bergerat, A., de Massy, B., Gadelle, D., Varoutas, P.C., Nicolas, A., and Forterre, P. (1997). An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature *386*, 414–417.

Beretta, G.L., Binaschi, M., Zagni, E., Capuani, L., and Capranico, G. (1999). Tethering a type IB topoisomerase to a DNA site by enzyme fusion to a heterologous site-selective DNA-binding protein domain. Cancer Res. *59*, 3689–3697.

Borde, V., Wu, T.C., and Lichten, M. (1999). Use of a recombination reporter insert to define meiotic recombination domains on chromosome III of *Saccharomyces cerevisiae*. Mol. Cell. Biol. *19*, 4832–4842.

Borde, V., Goldman, A.S., and Lichten, M. (2000). Direct coupling between meiotic DNA replication and recombination initiation. Science *290*, 806–809.

Bullard, S.A., Kim, S., Galbraith, A.M., and Malone, R.E. (1996). Double strand breaks at the *HIS2* recombination hot spot in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *93*, 13054–13059.

Cha, R.S., Weiner, B.M., Keeney, S., Dekker, J., and Kleckner, N. (2000). Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev. *14*, 493–503.

Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., and Herskowitz, I. (1998). The transcriptional program of sporulation in budding yeast. Science *282*, 699–705.

de Vries, H. (1903). Befruchtung und Bastardierung (Leipzig). English translation by C.S. Gager entitled Fertilization and Hybridization in Intracellular pangenesis (Chicago: Open Court Publ. Co), pp. 217–263.

Diaz, R.L., Alcid, A.D., Berger, J.M., and Keeney, S. (2002). Identification of residues in yeast Spo11p critical for meiotic DNA double-strand break formation. Mol. Cell. Biol. *22*, 1106–1115.

Dirick, L., Goetsch, L., Ammerer, G., and Byers, B. (1998). Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science *281*, 1854–1857.

Fan, Q.Q., and Petes, T.D. (1996). Relationship between nuclease-hypersensitive sites and meiotic recombination hot spot activity at the *HIS4* locus of *Saccharomyces cerevisiae*. Mol. Cell. Biol. *16*, 2037–2043.

Forsburg, S.L. (2002). Only connect: linking meiotic DNA replication to chromosome dynamics. Mol. Cell *9*, 703–711.

Gerton, J.L., DeRisi, J., Shroff, R., Lichten, M., Brown, P.O., and Petes, T.D. (2000). Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *97*, 11383–11390.

Goffeau et al. (1997). The yeast genome directory. Nature Suppl. *387*, 1–105.

Huibregtse, J.M., Good, P.D., Marczynski, G.T., Jaehning, J.A., and Engelke, D.R. (1993). Gal4 protein binding is required but not sufficient for derepression and induction of *GAL2* expression. J. Biol. Chem. *268*, 22219–22222.

Jasin, M. (1996). Genetic manipulation of genomes with rare-cutting endonucleases. Trends Genet. *12*, 224–228.

Johnston, M. (1987). A model fungal gene regulatory mechanism: the GAL genes of *Saccharomyces cerevisiae*. Microbiol. Rev. *51*, 458–476.

Kane, S.M., and Roth, R. (1974). Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. *118*, 8–14.

Keeney, S. (2001). The mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. *52*, 1–53.

Keeney, S., and Kleckner, N. (1996). Communication between homologous chromosomes: genetic alterations at a nuclease-hypersensitive site can alter mitotic chromatin structure at that site both in *cis* and in *trans*. Genes Cells *1*, 475–489.

Keeney, S., Giroux, C.N., and Kleckner, N. (1997). Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell *88*, 375–384.

Kim, Y.G., Smith, J., Durgesha, M., and Chandrasegaran, S. (1998). Chimeric restriction enzyme: Gal4 fusion to FokI cleavage domain. Biol. Chem. *379*, 489–495.

Klapholz, S., Waddell, C.S., and Esposito, R.E. (1985). The role of the *SPO11* gene in meiotic recombination in yeast. Genetics *110*, 187–216.

Lee, J.Y., and Orr-Weaver, T.L. (2001). The molecular basis of sister-chromatid cohesion. Annu. Rev. Cell Dev. Biol. *17*, 753–777.

Lichten, M., and Goldman, A.S. (1995). Meiotic recombination hotspots. Annu. Rev. Genet. *29*, 423–444.

Malkova, A., Klein, F., Leung, W.Y., and Haber, J.E. (2000). HO endonuclease-induced recombination in yeast meiosis resembles Spo11-induced events. Proc. Natl. Acad. Sci. USA *97*, 14500–14505.

Moore, C.S., Lee, J.S., Birren, B., Stetten, G., Baxter, L.L., and Reeves, R.H. (1999). Integration of cytogenetic with recombinational and physical maps of mouse chromosome 16. Genomics *1*, 1–5.

Morgan, T.H., and Cattell, E. (1912). Data for the study of sex-linked inheritance in *Drosophila*. J. Exp. Zool. *13*, 79–101.

Mozo, T., Dewar, K., Dunn, P., Ecker, J.R., Fischer, S., Kloska, S., Lehrach, H., Marra, M., Martienssen, R., Meier-Ewer, S., and Altmann, T. (1999). A complete BAC-based physical map of the *Arabidopsis thaliana* genome. Nat. Genet. *22*, 271–275.

Neale, M., Ramachandran, M., Trelles-Sticken, E., Scherthan, H., and Goldman, A.S. (2002). Wild-type levels of Spo11-induced DSBs are required for normal single-strand resection during meiosis. Mol. Cell *9*, 835–846.

Noor, M.A., Cunningham, A.L., and Larkin, J.C. (2001). Consequences of recombination rate variation on quantitative trait locus mapping studies. Simulations based on the *Drosophila melanogaster* genome. Genetics *159*, 581–588.

Ohta, K., Shibata, T., and Nicolas, A. (1994). Changes in chromatin structure at recombination initiation sites during yeast meiosis. EMBO J. *13*, 5754–5763.

Ohta, K., Wu, T.C., Lichten, M., and Shibata, T. (1999). Competitive inactivation of a double-strand DNA break site involves parallel suppression of meiosis-induced changes in chromatin configuration. Nucleic Acids Res. *27*, 2175–2180.

Petes, T.D. (2001). Meiotic recombination hot spots and cold spots. Nat. Rev. Genet. *2*, 360–369.

Ren, B., Robert, F., Wyrick, J., Aparicio, O., Jennings, E., Simon, I., Zeitlinger, J., Schreiber, J., Hannett, N., Kanin, E., et al. (2000). Genome-wide location and function of DNA binding proteins. Science *290*, 2306–2309.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Smith, K.N., and Nicolas, A. (1998). Recombination at work for meiosis. Curr. Opin. Genet. Dev. *8*, 200–211.

Smith, K.N., Penkner, A., Ohta, K., Klein, F., and Nicolas, A. (2001). B-type cyclins *CLB5* and *CLB6* control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr. Biol. *11*, 88–97.

Steiner, W.W., Schreckhise, R.W., and Smith, G.R. (2002). Meiotic DNA breaks at the *S. pombe* recombination hot spot M26. Mol. Cell *9*, 847–855.

Stuart, D., and Wittenberg, C. (1998). *CLB5* and *CLB6* are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. *12*, 2698–2710.

Sturtevant, A.H. (1913). The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. J. Exp. Zool. *14*, 43–59.

Sun, H., Treco, D., Schultes, N.P., and Szostak, J.W. (1989). Double-strand breaks at an initiation site for meiotic gene conversion. Nature *338*, 87–90.

Vedel, M., and Nicolas, A. (1999). *CYS3*, a hotspot of meiotic recombination in *Saccharomyces cerevisiae*: effects of heterozygosity and

mismatch repair fuctions on gene conversion and recombination intermediates. Genetics *151*, 1245–1259.

Xu, F., and Petes, T.D. (1996). Fine-structure mapping of meiosis specific double-strand DNA breaks at a recombination hotspot associated with an insertion of telomeric sequences upstream of the *HIS4* locus in yeast. Genetics *143*, 1115–1125.

Wach, A. (1996). PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *Saccharomyces cerevisiae*. Yeast *12*, 259–265.

Whitehouse, H.L.K. (1982). Genetic recombination. Understanding the Mechanisms (New York: Wiley-Interscience).

Wu, T.-C., and Lichten, M. (1994). Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science *263*, 515–518.

Wu, T.-C., and Lichten, M. (1995). Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics *140*, 55–66.

Yu, A., Zhao, C., Fan, Y., Jang, W., Mungall, A.J., Deloukas, P., Olsen, A., Doggett, N.A., Ghebranious, N., Broman, K.W., and Weber, J.L. (2001). Comparison of human genetic and sequence-based physical maps. Nature *409*, 951–953.

Zenvirth, D., Arbel, T., Sherman, A., Goldway, M., Klein, S., and Simchen, G. (1992). Multiple sites for double-strand breaks in whole meiotic chromosomes of *Saccharomyces cerevisiae*. EMBO J. *11*, 3441–3447.

Zickler, D., and Kleckner, N. (1998). The leptotene-zygotene transition of meiosis. Annu. Rev. Genet. *32*, 619–697.

Zickler, D., and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. *33*, 603–754.